Short Communication

Bulky DNA Adducts and Risk of Cancer: A Meta-Analysis[1]

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Abstract

We present a meta-analysis to test the hypothesis that the presence of a high level of bulky DNA adducts in tissues is associated with an increased risk of cancer in humans. Seven articles were selected that matched the selection criteria, for a total of 691 cancer patients and 632 control subjects. In five studies the cases had lung cancer, in one oral cancer, and in one bladder cancer. Six studies measured adducts in WBCs and one in normal lung tissue around tumor tissue. Six were case-control investigations, and one was a case-control study on lung cancer nested within a cohort. Current smokers showed a statistically significant difference between cases and controls, with cases having 83% higher levels of adducts than controls (95% confidence interval, 0.44–1.22). Results were negative or contradictory in ex-smokers and nonsmokers. This observation was confirmed by sensitivity analyses. Publication bias does not seem to be a problem. Despite some methodological limitations, our meta-analysis shows that current smokers with high levels of adducts have an increased risk of lung and bladder cancers. This conclusion also suggests that similar (aromatic) compounds may be involved in the etiology of both types of cancer.

Introduction

"Bulky" DNA adducts represent an integrated marker of exposure to aromatic compounds, and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage. The level of bulky adducts in WBCs has been shown to correlate with external exposure to PAHs[3] in a few investigations. The association with tobacco smoke was inconsistent (1–4). Adducts were negatively associated with the consumption of fruit and vegetables (5–7), possibly through a stimulation of DNA repair (8). Their biological meaning for carcinogenesis has been illustrated in a few elegant experiments. Denissenko *et al.* (9) have shown that the main metabolite of benzo(*a*)pyrene forms adducts in the same codon of the *p53* gene where characteristic mutations are found in the lungs of smokers. In animal experiments, PAH-DNA adduct levels explained tumor outcome. For example, Nesnow *et al.* (10) found a correlation between the formation of DNA adducts by PAHs, and the induction of *ras* mutations and lung tumor in mice.

The purpose of the present meta-analysis is to test the hypothesis that the presence of a high level of bulky DNA adducts in tissues is associated with an increased risk of cancer in humans.

Materials and Methods

The Medline database was searched for the period between January 1990 and March 2002, supplemented with manual bibliography review. We collected all of the studies conforming to the following criteria: (*a*) case-control or cohort studies comparing bulky DNA adduct levels in cancer patients and control subjects; and (*b*) separate comparisons for current, former, and never-smokers.

We excluded specific adducts such as those formed by aflatoxin or cytostatic drugs.

Seven articles were selected that matched the selection criteria, for a total of 691 cancer patients and 632 control subjects. In five studies the cases had lung cancer (11–15), in one oral cancer (16), and in one bladder cancer (17). Bulky DNA adducts were measured by ^{32}P-postlabelling (in the majority of studies) or ELISA. Six studies measured adducts in WBCs and one (12) in normal lung tissue around tumor tissue. Six were case-control investigations, and one was a case-control study on lung cancer nested within a cohort (15).

Quality of the Studies. We used three criteria to evaluate the quality of the studies, and assigned scores on this basis (1 lowest, 3 highest; score 1 if information unavailable): (*a*) population- or hospital-based study; (*b*) response rate; and (*c*) blinding of procedures and consideration of confounders. Results of assessment are: Popp A2 B1 C2, average 1.7; Tang 1995 A2 B3 C3, average 2.7; Hou A3 B3 C3, average 3; Cheng A1 B1 C2, average 1.3; Peluso A2 B3 C2, average 2.3; Vulimiri A2 B1 C2, average 1.7; Tang 2001 A3 B3 C3, average 3.

Analysis. Average adduct levels differed markedly among studies, both in cases and controls, ranging from 0.4×10^8 to 7.9×10^8 in the WBC of controls. This may be partially attributable to interlaboratory variability, and partially to the different methods of blood collection and storing. The results were standardized by dividing within each study all of the means and SDs by the average of the control groups. Therefore, standardized control means were set to one in all of the studies.

Meta-analysis was carried out using the RevMan 4.1 software, available through the Cochrane Library.[4] Heterogeneity among studies was tested with Breslow-Day's test (18), and a random effect model was used in the meta-analysis to account for interstudy variability (19). We have computed standardized

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; WMD, weighed mean difference; CI, confidence interval; RAL, relative adduct level.

[4] Internet address: http://www.york.ac.uk/inst/crd/cochlib.htm.

Table 1 Meta-analysis of studies on bulky DNA adducts and cancer, random effect model, current smokers only

Study	Tumour n	Tumour mean(sd)	Control n	Control mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Popp 1993	20	0.57(0.80)	11	1.00(1.63)		8.4	-0.43[-1.46,0.60]
Tang 1995	52	2.08(1.83)	25	1.00(0.65)		14.2	1.08[0.52,1.64]
Hou 1999	35	1.00(0.84)	44	1.00(0.73)		17.2	0.00[-0.35,0.35]
Vulimiri 2000	34	2.11(0.37)	29	1.00(0.13)		19.5	1.11[0.98,1.24]
Peluso 2000	94	2.25(3.89)	27	1.00(1.43)		9.1	1.25[0.30,2.20]
Cheng 2000	38	2.59(1.62)	22	1.00(0.94)		12.9	1.59[0.94,2.24]
Tang 2001	36	1.96(0.72)	64	1.00(0.09)		18.6	0.96[0.72,1.20]
Total(95%CI)	309		222			100.0	0.83[0.44,1.22]

Test for heterogeneity chi-square=44.56 df=6 p<0.00001
Test for overall effect z=4.16 p=0.00003

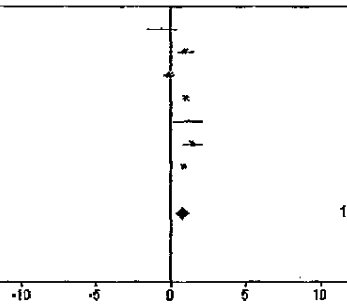


Table 2 Meta-analysis of studies on bulky DNA adducts and cancer, random effect model, former smokers only

Study	Tumour n	Tumour mean(sd)	Control n	Control mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Tang 1995	58	1.67(2.71)	34	1.00(0.98)		15.9	0.67[-0.10,1.44]
Hou 1999	54	1.10(1.02)	30	1.00(0.75)		43.1	0.10[-0.28,0.48]
Vulimiri 2000	6	2.18(5.34)	5	1.00(0.62)		0.6	1.18[-3.13,5.49]
Peluso 2000	42	0.66(0.54)	24	1.00(2.04)		14.0	-0.34[-1.17,0.49]
Tang 2001	35	0.72(0.86)	64	1.00(1.96)		26.4	-0.28[-0.84,0.28]
Total(95%CI)	195		157			100.0	0.04[-0.30,0.37]

Test for heterogeneity chi-square=4.98 df=4 p=0.29
Test for overall effect z=0.21 p=0.8

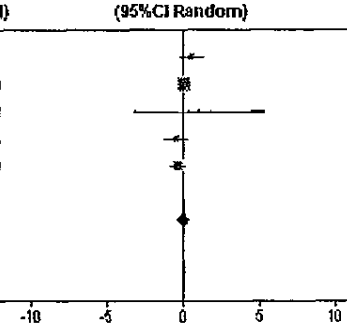
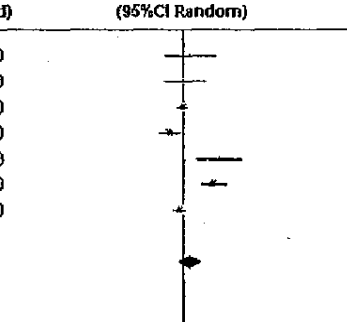


Table 3 Meta-analysis of studies on bulky DNA adducts and cancer, random effect model, never-smokers only

Study	Tumour n	Tumour mean(sd)	Control n	Control mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Popp 1993	3	1.54(1.25)	4	1.00(1.03)		9.2	0.54[-1.20,2.28]
Tang 1995	9	1.18(2.00)	39	1.00(1.36)		11.5	0.18[-1.19,1.55]
Hou 1999	82	1.00(0.88)	72	1.00(1.18)		18.8	0.00[-0.33,0.33]
Vulimiri 2000	3	0.16(0.04)	13	1.00(1.28)		16.6	-0.84[-1.54,-0.14]
Peluso 2000	22	3.42(3.51)	55	1.00(1.32)		10.6	2.42[0.91,3.93]
Cheng 2000	32	3.07(2.33)	11	1.00(0.53)		15.3	2.07[1.20,2.94]
Tang 2001	15	0.80(0.64)	30	1.00(1.05)		17.9	-0.20[-0.70,0.30]
Total(95%CI)	166		224			100.0	0.47[-0.26,1.19]

Test for heterogeneity chi-square=37.96 df=6 p<0.00001
Test for overall effect z=1.27 p=0.2



WMDs between cases and controls in each study, and the overall WMD. For each WMD we computed 95% CIs.

Results

The results are reported in Tables 1–3, according to smoking habits (current, former, and never-smoker). Current smokers show a statistically significant difference between cases and controls with cases having 83% higher levels of adducts than controls. Of seven studies, five are consistent in reporting a difference, including the only cohort study, and each of the five is statistically significant. No association between adduct levels and the case status is observed in former smokers (Table 2), with complete consistency among investigations. Results on nonsmokers are inconsistent; only two studies show a statisti-

cally significant positive difference between cases and controls (one based on the measurement of adducts in the lung tissue, Ref. 12; and one based on measurements in WBC in bladder cancer cases and controls, Ref. 17). Overall, the WMD for never-smokers is 47%, not statistically significant and almost entirely attributable to the two studies mentioned above.

Sensitivity Analysis. If we exclude the papers with a quality score < 2, we still have in Table 1 three positive results of 4 (the exception being Hou).

Another type of sensitivity analysis includes only studies on lung cancer and excludes the investigation that measured adducts in lung tissue. In this case the standardized difference for RAL is 0.79 (95% CI, 0.34–1.24) for current smokers, 0.10 (95% CI, −0.29–0.49) for former smokers, and −0.21 (95% CI, −0.58–0.15) for nonsmokers, results similar to those obtained in the overall analysis.

Discussion

We have identified seven studies that have considered the association between cancer at different sites and the levels of "bulky" DNA adducts, according to smoking status. An overall 83% excess of adduct levels was found in cases compared with controls in current smokers (95% CI, 44–122%). No association was found among former smokers, whereas never-smokers showed very heterogeneous results. These observations are in accordance with the findings of the only prospective study available (15), which also found that DNA adduct levels measured in WBCs were predictive of lung cancer occurrence only in current smokers.

The interpretation of the meta-analysis is limited by the fact that in case-control studies the biomarker may reflect the disease rather than the etiology. However, an exception is represented by the cohort study already mentioned (15). The importance of that study rests on the measurement of adducts in blood samples that were collected years before cancer onset, thus ruling out the possibility that the higher adduct levels were because of the metabolic changes associated with an already existing cancer.

Bulky DNA adducts represent exposure to PAH and other aromatic compounds after the action of metabolizing enzymes; they are in steady-state if exposure is constant. In addition, they also reflect the action of DNA repair enzymes and, thus, of individual susceptibility. The role of individual susceptibility related to DNA repair is indirectly suggested by the observation that the lymphocytes of cancer patients (and of their healthy relatives) show higher levels of DNA adducts when treated with electrophilic chemicals compared with lymphocytes of noncancerous individuals (20).

We also have to consider the limitations of our explanatory model. In particular, the level of measurement error for bulky adducts is not well known but seems to be high (coefficient of variation around 20–30%). However, the effect of measurement error is to attenuate a relationship if error is evenly distributed in the comparison groups (21). So, measurement error is expected to blur existing associations rather than to reveal false associations.

An important observation that requires explanation is the presence of higher adduct levels among cases, in comparison with controls, only in current smokers. Former smoker was only episodically defined in the studies; heterogeneity in the definition might have blurred the association with adducts in former smokers. An alternative explanation is that only current smokers have the relevant exposure, and the difference between cases and controls is the expression of genetic predisposition of cancer patients, possibly related to the ability in the repair of DNA damage. It should be stressed that the measurement of adducts in WBCs only indirectly refers to changes in the target tissues (lung or bladder). This is an obvious limitation in the interpretation of the present epidemiological findings.

We have observed interstudy variation in the levels of adducts that were detected; however, such variation was considerably attenuated by normalizing the measures on the basis of the levels in controls. The remaining variation may be attributable partially to interlaboratory variability and partially to the different sources of DNA used (tumor tissue or peripheral blood lymphocytes). Although an effort to compare and standardize laboratory procedures has been undertaken and published by a group of European researchers (22), more standardized measurements are needed in future investigations.

Publication bias could justify the findings if small positive studies have greater chances of being published than small negative studies. However, there is no evidence of an association between the study size and the results: negative studies tend to be as small as the positive ones (Tables 1–3).

In conclusion, despite some methodological limitations, our meta-analysis shows that current smokers with high levels of adducts have an increased risk of lung and bladder cancers. This conclusion also suggests that similar (aromatic) compounds may be involved in the etiology of both types of cancer.

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